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Pt(II) complexes with (N,N′) or (C,N,E)$^-$ (E = N,S) ligands: Cytotoxic studies, effect on DNA tertiary structure and structure–activity relationships

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ABSTRACT

The cytotoxic activity of two series of platinum(II) complexes containing the polyfunctional imines R^1–CH=N–R^2 [R^1 = phenyl or ferrocenyl unit and $R^2 = (CH_2)_n$–CH_2–NMe_2 where $n = 1$ or 2) (**1** and **2**) or C_6H_4-2-SMe (**3**)] acting as a bidentate (N,N′) (**4**–**7**) or terdentate [C(phenyl or ferrocenyl),N,N′]$^-$ (**8**–**10**) or [C(ferrocenyl),N,S]$^-$ ligand (**11**) in front of A549 lung, MDA-MB231 breast and HCT116 colon human adenocarcinoma cell lines is reported. The results reveal that most of the platinum(II) complexes are active against the three assayed lines and compounds **6**, **7** and the platinacycles **10** and **11** exhibit a remarkable antiproliferative activity, even greater than *cisplatin* itself, in the *cisplatin* resistant HCT116 human cancer cell line. Electrophoretic DNA migration studies showed that most of them modify the DNA tertiary structure in a similar way as the reference *cisplatin*. Solution studies of a selection of the most relevant complexes have also been performed in order to test: (a) their stability in the aqueous biological medium and/or the formation of biologically active species and (b) their proclivity to react with 9-methylguanine (9-MeG), as a model nucleobase. Computational studies at DFT level have also been performed in order to explain the different solution behaviour of the complexes and their proclivity to react with the nucleobase.

1. Introduction

The potential of metal-based anticancer agents has been widely explored and recognised[1–3] since the landmark discovery of the biological activity of *cisplatin*.[4] To date this prototypical anticancer drug remains one of the most effective chemotherapeutic agents in clinical use.[5] However, its utility against cancer is limited by (a) severe dose-limiting toxicity, such as neuro-, hepato-, and nephrotoxicity[6–9] and/or (b) inherent or acquired resistance.[10]

A better understanding of the cellular response to *cisplatin* and how tumours are or become resistant,[11–14] will contribute to the design of novel platinum compounds[15] with improving anticancer properties. *Cisplatin* enters cells by passive diffusion[16] and also, as discovered later, by active transport mediated by the copper transporter Ctr1p in yeast and mammals.[17,18] Once inside the cell, the low chloride concentration (4–20 mM) results in drug aquation with the loss of one or both of the chlorido ligands. The resulting aquo-platinum complex binds to its target, DNA, by the formation of covalent cross-links; being the 1,2-intrastrand d(GpG) cross-link the major adduct. Binding of *cisplatin* to DNA causes significant distortion of the helical structure and results in inhibition of DNA replication and transcription,[16,19] which ultimately leads to cell cycle arrest and cellular apoptosis. The distorted, platinated DNA

Abbreviations: DMEM, Dulbecco's modified eagle medium; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; G, guanine; 9-MeG, 9-methylguanine; 9-AA, 9-aminoacridine; ELISA, enzyme-linked immunosorbent assay; DFT, density functional theory; B3LYP, Becke 3-parameter (exchange)—Lee, Yang and Parr (correlation) functional; LANL2DZ, Los Alamos National Laboratory 2 double zeta basis set; C-PCM, conductor polarized continuum model.

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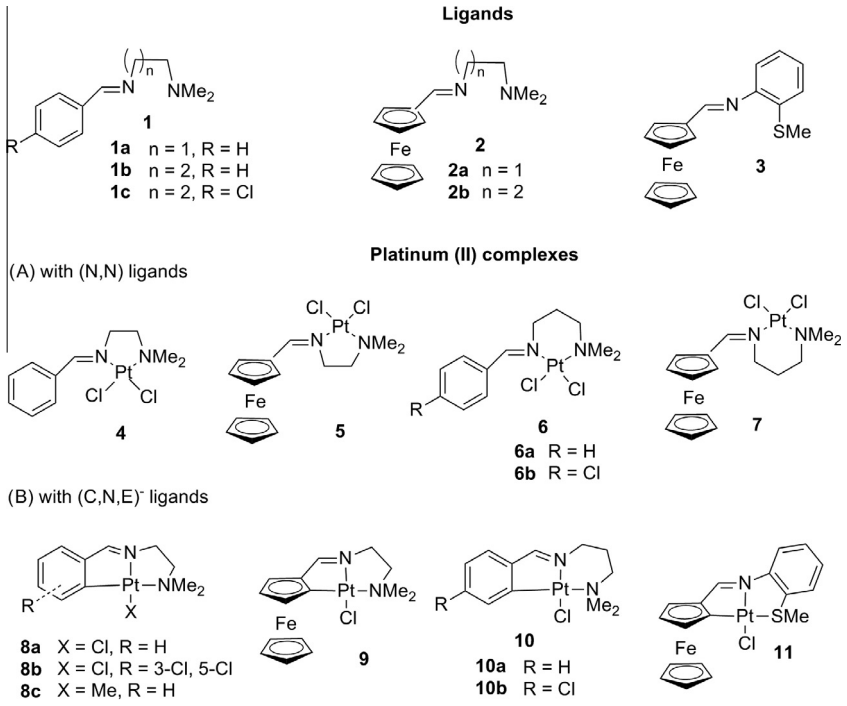


Figure 1. Chemical formulae of the free ligands (**1**–**3**) and the platinum(II) complexes (**4**–**11**) selected for this study.

Table 1
Cytotoxic activities (IC_{50} values[a]) on A549 lung, MDA-MB231 breast human and HCT116 colon cancer cell lines for the free ligands (**1a**–**1c** and **3**)[b] the platinum complexes (**4**–**11**) and *cisplatin*

Type of product	Mode of binding of the ligand	IC_{50}(μM) for the cell lines:		
		A549	MDA-MB231	HCT116
Ligand[b]				
1a	—	>100	>100	>100
1b	—	>100	>100	>100
1c	—	>100	>100	>100
3	—	173 ± 25	nd	44 ± nd
Platinum(II) complexes with bidentate ligands				
4	(N,N′)	26 ± 12	47 ± nd	35 ± 2.2
5	(N,N′)	>100	57 ± nd	55 ± 6.8
6a	(N,N′)	95 ± 7	14.1 ± 2.4	11.9 ± 9 2
6b	(N,N′)	>200	10.7 ± 4.6	10.6 ± 1.7
7	(N,N′)	178 ± 32	28 ± 0.3	30 ± 4.2
Platinum(II) complexes with terdentate ligands				
8a	[C(phenyl), N,N′]⁻	95 ± 7	10.7 ± 4.6	60 ± 1.7
8b	[C(phenyl), N,N′]⁻	38 ± 18	42.2 ± 9.5	>100
8c	[C(phenyl), N,N′]⁻	27.0 ± 10	30 ± 10.2	44 ± 8.5
9	[C(ferrocenyl), N,N′]⁻	75 ± 14	87 ± nd	45 ± 7.8
10a	[C(phenyl), N,N′]⁻	40 ± 14	28 ± 2.4	28 ± 6.7
10b	[C(phenyl), N,N′]⁻	23 ± 4	17 ± 1.1	18 ± 1.9
11	[C(ferrocenyl), N,S′]⁻	23 ± 8	32 ± 6.2	20.7 ± 3.8
Cisplatin	(N)	9.3 ± 3.0	6.5 ± 2.4	40 ± 4.4

[a] Data are shown as the mean values of two or more experiments performed in triplicate with the corresponding standard deviation (SD).
[b] Ref. 32.

structure also serves as a recognition binding site for cellular proteins,[11,12] such transcription factors, histones, high-mobility group (HMG)-domain proteins, and nucleotide excision repair (NER) proteins, which may modulate the *cisplatin* effectiveness and/or resistance.

Taking these findings into account, one of the strategies for developing new anticancer agents include the design of platinum complexes that bind to DNA in a fundamentally different manner than *cisplatin*. One of them consists in the synthesis of new organic compounds with one or more N atoms with donor abilities (i.e., amines, oximes, thiosemicarbazones, functionalized azoles, etc.) and their use as ligands for Pt(II).[20–23] Despite the great number of studies on this field and the variety of complexes reported in recent years, to the best of our knowledge, none of these studies deals with sets of platinum(II) complexes derived from closely related ligands, exhibiting different coordination modes. Structure–activity relationship (SAR) upon this sets of platinum(II) compounds may provide interesting information of how their cytotoxic activity could be influenced by several structural factors such as (a) the donor atoms of the ligand, (b) its mode of binding [i.e., (N),

(N,E), $(C,N,E)^-$, E = additional heteroatom], (c) the chelate size, (d) the presence of substituents or even (e) the nature of additional ancillary ligands.

During the last decade, we have prepared different families of polyfunctional imines (**1**–**3**, Fig. 1)[24–28] and used them as ligands to build up a wide variety of platinum(II) complexes (**4**–**11**),[24,26–31] in which the Schiff bases behave as neutral and bidentate (N,N′) ligands (in compounds **4**–**7** of Fig. 1) or as monoanionic and terdentate $(C,N,N')^-$ (in **8**–**10**) or $(C,N,S)^-$ (in **11**) ligands.

Now we report comparative studies of the cytotoxic activity of the platinum(II) complexes (**4**–**11**) in front of A549 lung, MDA-MB231 breast and HCT116 colon (*cisplatin* resistant) human adenocarcinoma cell lines, together with the investigation of their effect on the electrophoretic mobility of DNA. The results presented here provide conclusive evidences of the influence of the type of ligands and their binding mode on their biological effectiveness.

2. Results and discussion

2.1. Biological studies

2.1.1. Antiproliferative assay

Human lung, breast and colon cancer cell lines (A549, MDA-MB231 and HCT116, respectively) were used to test the cytotoxic activity of the Pt(II) complexes with (N,N′) ligands (**4**–**7**) and the platinacycles (**8**–**11**). For comparison purposes *cisplatin* (as positive control) and the free ligands **1**–**3** were evaluated under the same experimental conditions.[32]

Data presented in Table 1 shows that the investigated compounds exhibit variable selectivity in front of the adenocarcinoma cell lines tested (Table 1, Fig. 2). Several complexes showed greater cytotoxicity effectiveness than that of *cisplatin* versus the platinum resistant HCT116 cancer cell line selected, namely compounds **4**, **6a**, **6b**, **7**, **10a**, **10b**, and **11** (Fig. 3).

Regarding the chelated (N,N′) Pt(II) complexes (**4**–**7**), it has been reported that the five membered 1,2-ethanediamine complexes showed greater antiproliferative activity than the six membered 1,3-propanediamine complexes in front of L1210 cell line.[20,33] In our study, the chelated five-membered complex **4** exhibited greater potency than compound **6a** (six-membered chelate ring) in A549 lung cancer cell line. However, for the other cancer cell lines tested (MDA-MB231 breast and HCT116 colon) complex **4** showed higher IC_{50} values than that of complex **6a**. In *cisplatin* resistant HCT116 colon adenocarcinoma cell line, the six-membered complexes **6a** and **6b** exhibited a cytotoxic effectiveness approximately fourfold greater than that of *cisplatin*. It should be mentioned that a

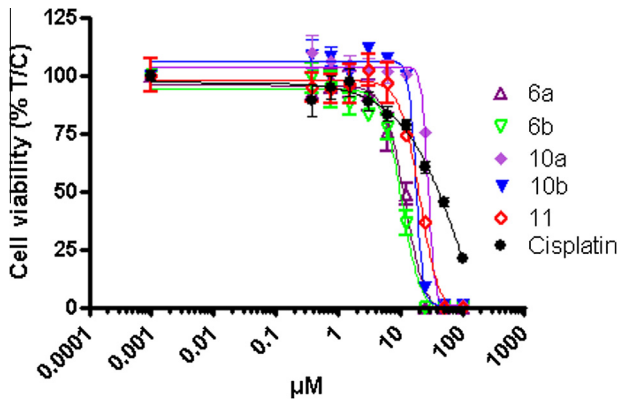


Figure 2. Inhibition of cell growth proliferation in HCT116 colon cancer cells line, after 72 h of exposure to the compounds **6** (**6a**–**6b**), **10** (**10a**–**10b**), **11** and *cisplatin*.

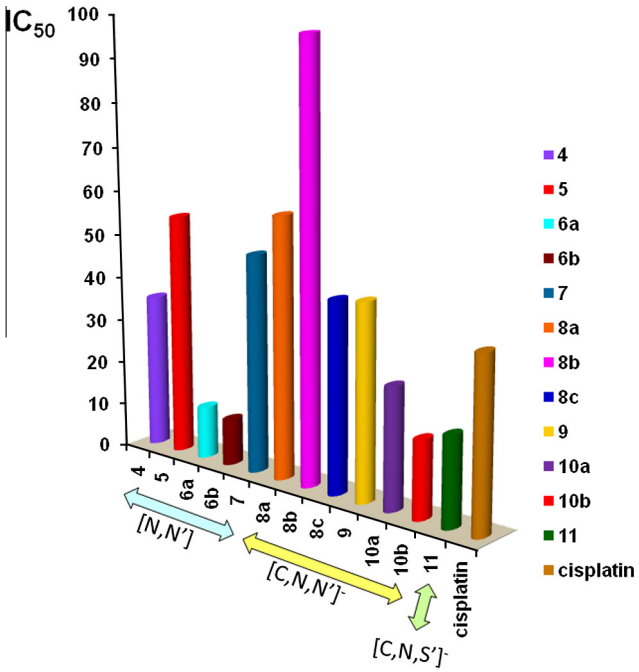


Figure 3. Comparison of the IC_{50} (μM) values obtained for the platinum(II) complexes with bidentate (N,N′) ligands (**3**–**7**), the cyclometallated compounds (**8**–**11**) and *cisplatin* in front of the HCT-116 cell line.

similar variable effect, depending of the assayed cancer cell line, has also been observed for Pt(II) complexes with 2,3-diaminopropanoate or 2,4-diaminobutiroate ligands towards A431, Hela and HL-60 cell lines.[33] Furthermore the ferrocene derivative **7** (six membered) exhibited greater cytotoxic effectiveness than the corresponding five membered ferrocene derivative **5** in the three cancer cells lines assayed. No increase in potency is observed when the ferrocenyl compounds **5** and **7** are compared with the phenyl complexes **4** and **6a** in pairs: **5**–**4** and **7**–**6a**.

Considering the cyclometallated complexes **8** (**8a**–**8b**) having a tricyclic [6.5.5] system, the non-substituted compound **8a** exhibits a slight greater cytotoxic activity than the 3,5-dichlorido derivative **8b** in A549 lung and HCT116 colon cancer cell lines; and the opposite situation is given for MDA-MB231 breast cancer cell line. Surprisingly, the platinacycle **8c**, having a methyl ligand instead of the Cl^- ligand showed lower IC_{50} values in the three human cancer cell lines than the corresponding monofuntional complex **8a**.

Finally, the cyclometallated complexes **10** (**10a** and **10b**) and **11** having a tricyclic [6.5.6] and tetracyclic [5.5.5.6] system, respectively, showed a remarkable cytotoxic effectiveness, although not so high as the seven-membered platinacycles previously investigated in our research group.[34,35] In *cisplatin* resistant HCT116 colon adenocarcinoma cell line, platinacycle **10b** exhibited a low IC_{50} value, being approximately 2.2-fold more potent than *cisplatin*. Considering the two cycloplatinated ferrocene derivatives **9** and **11**, it is noteworthy that complex **11**, with Pt–S bond exhibits lower IC_{50} than compound **9** with a Pt–N(amine) bond.

2.1.2. DNA migration studies

The effect of binding of the compounds investigated in this study on supercoiled DNA was determined by their ability to alter the electrophoretic mobility of pBluescript plasmid DNA: supercoiled closed circular (ccc) and open circular (oc) forms. The ccc form usually moves faster due to its compact structure, so it showed almost one band in gel. When the test compounds (**1**–**11**) were incubated with plasmid DNA at 37 °C, most of them could coordinate to the DNA molecule, which in some extend was

cleaved into fragments, and the brightness of band was diminished in gel.

Figure 4 shows the electrophoretic mobility of native pBluescript DNA (40 μg/mL) incubated with the selected compounds (**1**–**11**) ranging from 2.5 to 200 μM concentrations. To provide a basis for comparison, incubation of DNA with *cisplatin* and 9-AA was also performed using the same concentrations and conditions. As expected, at the lowest concentration (2.5 μM) assayed, *cisplatin* greatly altered the electrophoretic mobility of pBluescript DNA.

For the free ligands **1** (**1a**–**1c**) an increase in the presence of nicked relaxed forms without shifting the plasmid DNA mobility of supecoiled form was observed when increasing the concentration of the compounds. For the ferrocenyl ligand **3** almost no decrease in mobility was observed at any assayed concentration (Fig. 4A).

The chelated complexes having five (**4** and **5**) and six (**6a** and **6b**) membered rings greatly alter the electrophoretic mobility of plasmid DNA (Fig. 4B). When increasing the concentration of the platinum complex, the migration rate of the supercoiled band decreases until it comigrates with the nicked relaxed band. In these titration experiments, the coalescence point (defined as the amount of platinum complex that is necessary for complete removal of all supercoils from DNA) occurs at 75 μM concentration of **4** and **5** and at 10 μM concentration of **6a** and **6b**. At high concentration of complexes **4** and **5** (lanes 8 and 9) and **6** (**6a**–**6b**, lanes 5, 6 and 7), a strong unwinding of negative supercoiled DNA to positive supercoiled DNA was displayed in the electrophoretogram (Fig. 4B). The same effect was observed for *cisplatin*[36] (Fig. 4D, lanes 5 and 6). For the ferrocene complex **7** a slight decrease in plasmid DNA mobility was observed in a dose dependent manner.

Platinacycles **8** (**8a**–**8c**) greatly alter the mobility of plasmid DNA. For these three compounds (Fig. 4C) the coalescence point, obtained in the titration assay occurs at 10 μM concentration of **8a** and **8b**, and at 50 μM concentration of **8c**. The lower efficiency of complex **8c** than that of **8a** in removing the supercoils from DNA could be related to the nature of the ancillary ligand {Cl (in **8a**) or Me (in **8c**)}. It is well known that Pt–Cl bonds are more labile than Pt–C bonds, thus metallacycle **8a** is expected to be more reactive than the methyl derivative **8c**. At high concentration of complexes **8a**–**8b** (lanes 5, 6 and 7) and complex **8c** (lanes 7, 8 and 9), the strong unwinding of negative supercoiled DNA to positive supercoiled DNA was also observed. Regarding the ferrocene platinacycle **9** a weaker effect on the migration rate of supercoiled closed circular plasmid DNA in a dose dependent manner was displayed. Paradoxically, compound **9** (ferrocenylimine) exhibits a similar structure as complex **8a** (benzylimine), which is able to produce a great unwinding effect on DNA.

The Pt(II) complexes **10a** and **10b** greatly alter the electrophoretic mobility of plasmid DNA (Fig. 2D). In the unwinding experiment the coalescence point for complexes **10a** and **10b** takes place with 5 and 25 μM, respectively. Interestingly these two compounds are even more potent than *cisplatin* in HCT116 colon

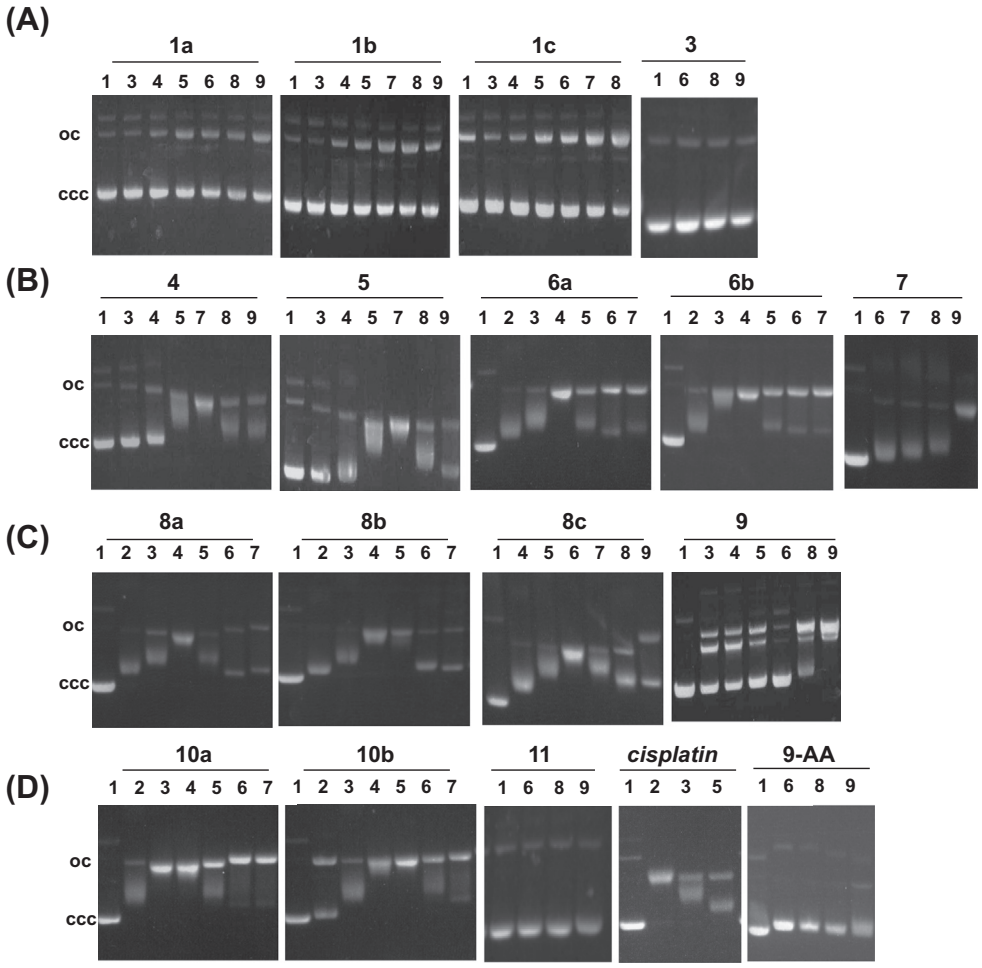


Figure 4. Interaction of pBluescript SK+ plasmid DNA (40 μg/ml) with increasing concentrations of: (A), free ligands (**1**–**3**), (B) their platinum(II) derivatives with (N,N′) ligands (**4**–**7**), and (C–D) 5-membered metallacycles (**8**–**11**) and *cisplatin* and 9-AA used for comparison purposes. Lanes 1, DNA only; lanes 2, 2.5 μM compound; lanes 3, 5 μM compound; lanes 4, 10 μM compound; lanes 5, 25 μM compound; and lane 6, 50 μM compound; lanes 7, 75 μM compound; lanes 8, 100 μM compound; and lane 9, 200 μM compound. [Abbreviations: ccc = supercoiled closed circular DNA form and oc = open circular DNA form].

cancer cell line (*cisplatin* resistant). With regard to the ferrocenyl-containing platinacycle **11**, although it exhibits a strong antiproliferative activity versus HCT116 adenocarcinoma cell line, no changes on the plasmid DNA migration rate were observed in the electrophoretogram. Compound **11**, with a polycyclic [5.5.5.6] system, is hypothesized to behave as 9-AA, which is used as intercalator reference in the experiment, or through another target/mechanism.

In general, the five-membered platinacycles under study exhibit a greater effect on DNA migration than that of the non-planar seven-membered platinacycle previously investigated in our group.[34,35] In recent studies centered on five-membered cyclometallated complexes, the metallacycle showed a planar five-membered ring structure[37–44] and hence it was hypothesized that they may act by intercalating into DNA.[41–44] However, the five-membered platinacycles **8** (**8a**–**8c**) and **10** (**10a**–**10b**) investigated in this study greatly alter the superhelicity of the DNA molecules in a similar way as the model *cisplatin*.

2.2. Solution studies

Given that the assayed compounds were insoluble in water, their stabilities in the aqueous biological media were evaluated, recording the ^{1}H NMR spectra of the solubilized compounds (**6a**, **8a**, **8c**, and **11**) in a mixture of DMSO-d_6 and D_2O (1:1). Then, the ^{1}H NMR spectra of the freshly prepared samples was compared with those obtained after different storage periods (Figs. S1–S4).

As shown in Figures S2 and S3 the solution behavior of **8a** and **8c** with identical terdentate ligands were markedly different. While **8c** was stable in aqueous solution for 48 h, for **8a**, a new compound was formed upon addition of D_2O. An analogous behavior to that of **8a** in D_2O was observed for the coordination compound **6a**, while for complex **11** no significant changes in the ^{1}H NMR spectra were detected after four days.

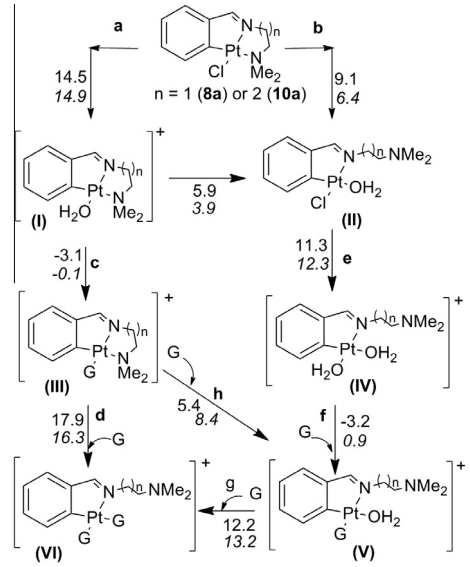
In a further step we also studied the effect produced by the presence of equimolar amounts of 9-methylguanine on the complexes solutions (Figs. S5–S7). In contrast with the results obtained above, in the presence of the nucleobase, complex **11** degradated (Fig. S7); whereas for compound **10a** (Fig. S6) the spectral changes detected were consistent with the coordination of 9-methylguanine to platinum. For **8c**, the quality of the ^{1}H NMR spectra decreased with time, but new signals were detected (in the range $7.7 < \delta < 8.4$ ppm) after 24 h of storage (Fig. S5-C).

2.3. Theoretical interpretation

It is well-known that the mechanism of action of *cisplatin* involves aquation prior to the binding to DNA[12–14] and we have proved that the length of the aliphatic chain (in **8a** and **10a**) and the nature of the ancillary ligand {Cl^- (in **8a**) or Me (in **8c**)} affects their cytotoxic activity, their effect on the electrophoretic mobility of DNA and their solution behavior. In view of this, and in order to compare the effect produced by the size of the chelate (in **8a** and **10a**) in the aquation process and the subsequent formation of the mono- or bis (adducts) with a nucleobase such as guanine, we undertook computational studies to determine the variations of the free energy (ΔG) of the process involving the incorporation of a H_2O molecule in the coordination sphere of the Pt(II), by either a simple ligand exchange reaction (Scheme 1, **a**) or the cleavage of the Pt–N(amine) bond and the coordination of the H_2O molecule (Scheme 1, **b**) to give the neutral species (**II**).

In a first stage the geometries of the complexes were optimized, afterwards the free energy and solvation corrections were calculated and finally we determined the ΔG values in water of each one of the reactions under study. Data presented in Scheme 1, show that differences ΔG (path **a**)—ΔG (path **b**) are 5.4 (for **8a**) and 8.5 kcal/mol (for **10a**). These values indicate that from a thermodynamic point of view the aquation is expected to proceed through path **b**. In addition, for **8a** the ΔG (for the formation of type **II** complex) is (ca. 2.7 kcal/mol) greater than for **10a**; thus suggesting that **10a** is more prone to give the aquo-complex **II** than **8a**.

It should be noted that for complex **II** with $n = 2$ the ΔG value for the aquation of the Pt–Cl bond and the formation of the monoadduct with guanine (**V**) (steps **e** and **f**), is greater than for its analogue with $n = 1$. These differences could be attributed to the



Scheme 1. Sequences of reactions chosen in the computational studies undertaken to the evaluate the aquation processes and the subsequent formation of the Pt(II)–guanine complexes {the mono-(**III**) or **V**) or bis (**VI**) aduct. The calculated ΔG value (in kcal/mol) of each one of the reactions, (in H_2O at 298 K) is presented next or close to the arrows and the values in itallics correspond to complexes with $n = 2$.

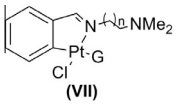
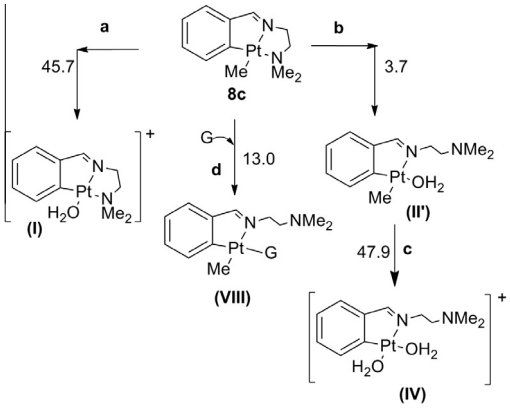


Figure 5. Guanine–platinum(II) monoadducts (**VII**) used in the computational studies.



Scheme 2. Sequences of reactions choosen for the computational studies for the evaluation of the aquation processes of the cycloplatinated complex **8c**. Transformation of the species formed (**I** and **II**′) into the mono- or bis-Pt(II)-guanine adducts would proceed as shown in Scheme 1, {from **I**: steps; **c** or (**c** and **h**); from **II** or **II**′: **e** and **f** to the monoadducts aducts **II**, and **V**, or step **g** for the bis(guanine) derivative}. The calculated ΔG for these processes (in kcal/mol) are presented next to the arrow.

presence of a larger and more flexible pendant arm. Furthermore, the computational studies also showed that the reactions of **8a** or **10a** with the guanine to give the monoadducts **VII**, shown in Figure 5, are even less favored than steps **a** and **b** of Scheme 1.

In order to clarify the role of the ancillary ligand {Cl (in **8a**) or Me (in **8c**)}, a parallel study on the aquation process of **8c** was also performed. As shown in Scheme 2, (steps **a** and **b**), the formation of **I** (in **a**) is less likely to occur than that of **II′** (by a ring opening process and coordination of water in step **b**). The ΔG for the following step **c** is more than four times higher than for the chlorido-complexes (**8a**). A similar result was obtained for the formation of the mono- or bis(guanine) adducts. These findings suggest that in **II′** the ancillary Me ligand inhibits the binding of the nucleobase. It should be noted that for the direct conversion of **8c** into the guanine monoadduct (**VIII** in Scheme 2, **d**) ΔG (13 kcal/mol) is smaller than that of the transformation of **II′** into the aquo-guanine derivative **V**—shown in Scheme 1—(44.7 kcal/mol).

3. Conclusions

The evaluation of the in vitro cytotoxic activity of five Pt(II) complexes with bidentate (N,N′) ligands (**4**–**7**) and eight 5-membered platinacycles with $(C,N,E)^-$ pincer ligands (**8**–**11**) revealed that all of them exhibit growth inhibitory activity against lung (A549), colon (HCT116) and breast (MDA-MB231 and MCF7) human cancer cell lines. For SAR analysis some preliminary conclusions could be highlighted when comparing the framework and the substitution pattern of the tested compounds: (1) the investigated compounds showed a variable selectivity towards the selected human adenocarcinoma cell lines assayed. For instance the chelated 1,3-propanediamine Pt(II) complexes **6** (**6a**–**6b**) exhibited four-times greater antiproliferative activity than that of *cisplatin* in HCT116 colon (*cisplatin* resistant) cancer cell line and no activity in A549 lung cancer cell line; (2) the [6.5.6] fused polycylic complexes **10** (**10a**–**10b**) and the [5.5.5.6] fused polycyclic complex **11** showed greater cytotoxic effectiveness than the corresponding [6.5.5] fused polycylic complexes **8** and [5.5.5] fused polycylic complex **9**; in particular complex **10b** inhibits cell growth proliferation at the level two times higher to that of *cisplatin* in HTC116 (*cisplatin* resistant) colon cancer cell line; (3) the substitution of a phenyl by a ferrocenyl group in compounds **4**, **6a** and **8a** to give complexes **5**, **7** and **9**, respectively did not show any increase in cytotoxicity; (4) the presence of a non-labile methyl ligand in **8c**, instead of the labile chlorido-ligand in **8a** interestingly give rise to an increase in potency.

Most of the complexes evaluated in this study show an effect on DNA electrophoretic mobility. Complexes **6**, **8** and **10** are those exhibiting the strongest interaction with DNA and display moderate to good cytotoxic activities towards the three cancer cell lines assayed. Solution studies and DFT calculations suggest that the replacement of the Me in (**8c**) by a Cl (in **8a**) is important as to modify the mode of action of the two products. Further studies on these areas are in progress centered on both (1) the mechanistic elucidation (cell cycle arrest, induction of apoptosis, etc) of the investigated complex, and (2) the development of more potent complex containing bidentate [(N,N′) or (N,S)], and terdentate [$(C,N,N')^-$ or $(C,N,S)^-$] donor ligands based on SAR analysis.

4. Experimental

4.1. Chemistry

4.1.1. Synthesis

The free ligands **1**–**3** and the platinum(II) complexes **4**–**11** were prepared according to procedures developed in our group[24–31] and characterized by NMR spectroscopy and X-ray crystallography (**3**–**4** and **7**, **8a**, **9**–**11**). The synthesis of the ligands **1**–**3** was accomplished by condensation reaction of equimolar amounts of the appropriate aldehyde and the corresponding amine in refluxing benzene. The synthesis was usually carried out using a Dean–Stark apparatus to remove the benzene–water azeotrope formed in the course of the reaction. Ligand **1a**[24] and **1b**–**1c**[25] were synthesized by reaction between the corresponding benzaldehide and the diamine $Me_2N(CH_2)_2NH_2$ or $Me_2N(CH_2)_3NH_2$. Ligands **2** (**2a**–**2b**)[26,28] were prepared by reaction between ferrocenecarbaldehyde and the appropriate diamine. Finally ligand **3**[27] was obtained by reaction of ferrocenecarbaldehyde with 2-mercaptoaniline. Complex **4**,[29] **5**,[26] **6** (**6a**–**6b**)[30] and **7**[28] were prepared by reaction of the corresponding ligand with *cis*-$[PtCl_2(DMSO)_2]$ in MeOH at reflux temperature. Complexes **8a**,[29] **9**,[26] **10** (**10a**–**10b**),[30] by reaction of the corresponding ligand with *cis*-$[PtCl_2(DMSO)_2]$/NaOAc in the same reaction conditions. Compound **8c**,[24] by reaction of ligand **1a** with $[Pt_2Me_4(\mu\text{-}SMe_2)_2]$ in acetone at reflux temperature. Complex **8b**[31] by reaction of a methyl precursor with CH_3COCl in CH_2Cl_2/MeOH at room temperature. Finally complex **11**,[27] by reaction of ligand **3** with *cis*-$[PtCl_2(PhCN)_2]$ in toluene at reflux temperature.

4.1.2. Study of the stability of the platinum(II) complexes in solution

These studies were performed using the following methodology: the corresponding complex [5.0 mg (of **6a**), 4.4 mg (of **8a**), 4.6 mg (of **8c**), or 5 mg (of **11**)] was introduced in a NMR tube, then 0.7 mL of a DMSO-d_6/D_2O (1:1) mixture were added, the tubes were sealed and the mixtures were shaken at 298 K to complete homogenization. The stability of the compounds in this solvent was studied by comparison of the ^1H NMR spectra of these freshly prepared solutions and those obtained after different periods of storage (t) at 298 K.

4.1.3. Study of the effect produced by 9-methylguanine on the platinum(II) complexes

A solution formed by the selected compound [4.4 mg (of **8c**), or 5.8 mg (of **10a**) or 4 mg (of **11**)] and 0.7 mL of a DMSO-d_6/D_2O (1:1) mixture was introduced in a NMR-tube. Then, the equimolar amount of 9-methylguanine was added, the resulting solution was shaken at 298 K to complete the homogenization and the tube was sealed. ^1H NMR spectra of these freshly prepared solutions were recorded at 298 K and the progress of the reaction was monitored by NMR after different periods of storage at 298 K.

4.2. Biological studies

4.2.1. Cell culture

Human lung A549, colon HCT116 cells and MBA-MD231 breast adenocarcinoma cells were grown as a monolayer culture in minimum essential medium (DMEM with L-glutamine, without glucose and without sodium pyruvate) in the presence of 10% heat-inactivated fetal calf serum, 10 mM D-glucose and 0.1% streptomycin/penicillin, in standard culture conditions (humidified air with 5% CO_2 at 37 °C).

4.2.2. Cell viability assay

For A549 cell viability assays, compounds were suspended in high purity DMSO at 20 mM as stock solution. To obtain final assay concentrations, they were diluted in DMEM (final concentration of DMSO was the same for all conditions and was always lower than 1%). The assay was performed by a variation of the MTT assay described by Mosmann[45] as specified by Matito et al.[46] which is based on the ability of live cells to cleave the tetrazolium ring of the MTT thus producing formazan, which absorbs at 550 nm. In brief, 3×10^3 A549 cells/well were cultured in 96 well plates for

24 h prior to the addition of the different compounds at different concentrations, in triplicate. After incubation for 72 h more, the supernatant was aspirated and 100 μL of filtered MTT (0.5 mg/mL) was added to each well. Following 1 h of incubation with the MTT, the supernatant was removed and the precipitated formazan was dissolved in 100 μL DMSO. Relative cell viability, compared to the viability of untreated cells, was measured by absorbance at 550 nm on an ELISA plate reader (Tecan Sunrise MR20-301, TECAN, Salzburg, Austria). Concentrations that inhibited cell growth by 50% (IC_{50}) after 72 h of treatment were subsequently calculated.

For MDA-MB231 and HCT116 cell viability assays, compounds were dissolved in high purity DMSO at 50 mM as stock solution. Then, serial dilutions were made with DMSO (1:1). In this way DMSO concentration in cell media was always the same. Finally, 1:500 dilutions of the serial dilutions of compounds on cell media were prepared. The assay was performed as described by Givens et al.[47] In brief, HCT116 and MDA-MB231 cells were plated at 5000 cells/well, respectively, in 100 μL media in tissue culture 96 well plates (Cultek). After 24 h, media was replaced by 100 μL/well of serial dilution of drugs. Control wells did not contain compounds. Each point concentration was run in triplicate. Reagent blanks, containing media and colorimetric reagent without cells were run on each plate. Blank values were subtracted from test values and were routinely 5–10% of uninhibited control values. Plates were incubated 72 h. Hexosaminidase activity was measured according to the following protocol: the media was removed and cells were washed once with PBS. 60 μL of substrate solution (*p*-nitrophenol-*N*-acetyl-β-D-glucosamide 7.5 mM, sodium citrate 0.1 M, pH 5.0, 0.25% Triton X-100) was added to each well and incubated at 37 °C for 1–2 h. After this incubation time, a bright yellow appears. Then, plates could be developed by adding 90 μL of developer solution (Glycine 50 mM, pH 10.4; EDTA 5 mM) and absorbance was recorded at 410 nm.

4.2.3. DNA migration studies

Compounds were dissolved in high purity DMSO at 10 mM as stock solution. Then, serial dilutions were made in milliQ water (1:1). Plasmid pBluescript SK+ (Stratagene) was obtained using a QIAGEN plasmid midi kit as described by the manufacturer. Interaction of drugs with pBluescript SK+ plasmid DNA was analyzed by agarose gel electrophoresis following a modification of the method described by Abdullah et al.[48] In brief, plasmid DNA aliquots (40 μg mL^{-1}) were incubated in TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.5) with different concentrations of the compounds (ranging from 0 μM to 100 μM) at 37 °C for 24 h. When indicated 200 μM concentration was also analyzed. Final DMSO concentration in the reactions was always lower than 1%. For comparison, *cisplatin* and 9-AA were used as reference controls. Aliquots of 20 μL of compound: DNA complexes containing 0.8 μg of DNA were subjected to 1% agarose gel electrophoresis in TAE buffer (40 mM Tris–acetate, 2 mM EDTA, pH 8.0). The gel was stained in the same buffer containing ethidium bromide (0.5 mg mL^{-1}) and visualized and photographed under UV light.

4.3. Computational studies

DFT calculations of all the platinum(II) complexes depicted in Schemes 1 and 2 and in Figure 5 have been performed at the B3LYP level[49,50] using the GAUSSIAN 03 software.[51] The basis set has been chosen as follows: LANL2DZ[52,53] for Pt and Cl, including polarization functions for Cl,[54] 6-31G[55] for hydrogen, and 6-31G(d) including polarization functions[55,56] for the remaining atoms. Solvent effects have been calculated on the optimized structures using the C-PCM model.[57]

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Supplementary data

Supplementary data (stability studies of the complexes (**6a**, **8a**, **8c**, and **11**) in solution, by comparison of the ^1H NMR spectra of freshly prepared samples and those obtained after different storage periods (Figs. S1–S4). Effect produced by the presence of equimolar amounts of 9-methylguanine on the complexes solutions (Figs. S5–S7)) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.05.005.

References and notes

1. Gasser, G.; Ott, I.; Metzler-Nolte, N. *J. Med. Chem.* **2011**, *54*, 3.
2. Dyson, P. J.; Sava, G. *Dalton Trans.* **2006**, 1929.
3. Thompson, K. H.; Orvig, C. *Dalton Trans.* **2006**, 761.
4. Rosenberg, B.; van Camp, L.; Krigas, T. *Nature* **1965**, *205*, 698.
5. Wheate, N. J.; Walker, S.; Craig, G. E.; Oun, R. *Dalton Trans.* **2010**, *39*, 8113.
6. Kannarkat, G.; Lasher, E. E.; Schiff, D. *Curr. Opin. Neurol.* **2007**, *20*, 719.
7. Markman, M. *Expert Opin. Drug Saf.* **2003**, *2*, 597.
8. Tsang, R. Y.; Al-Fayea, T.; Au, H.-J. *Drug Saf.* **2009**, *32*, 1109.
9. Sullivan, M. J. *Cancer* **2009**, *115*, 5623.
10. Köberle, B.; Tomicic, M. T.; Usanova, S.; Kaina, B. *BBA-Rev. Cancer* **2010**, *1806*, 172.
11. Jung, Y.; Lippard, S. J. *Chem. Rev.* **2007**, *107*, 1387.
12. Wang, D.; Lippard, S. J. *Nat. Rev. Drug Disc.* **2005**, *4*, 307.
13. Arnesano, F.; Natile, G. *Coord. Chem. Rev.* **2009**, *253*, 2070.
14. Gibson, D. *Dalton Trans.* **2009**, 10681.
15. Kelland, L. *Nat. Rev. Cancer* **2007**, *7*, 573.
16. Jamieson, E. R.; Lippard, S. J. *Chem. Rev.* **1999**, *99*, 2467.
17. Ishida, S.; Lee, J.; Thiele, D. J.; Herskowitz, I. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 14298.
18. Lin, X.; Okuda, T.; Holzer, A.; Howell, S. B. *Mol. Pharmacol.* **2002**, *62*, 1154.
19. Lee, K.-B.; Wang, D.; Lippard, S. J.; Sharp, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4239.
20. Montaña, Á. M.; Batalla, C. *Curr. Med. Chem.* **2009**, *16*, 2235.
21. Ling, E. C. H.; Allen, G. W.; Vickery, K.; Hambley, T. W. *J. Inorg. Biochem.* **2000**, *78*, 55.
22. Chellan, P.; Land, K. M.; Shokar, A.; Au, A.; An, S. H.; Clavel, C. M.; Dyson, P. J.; Kock, C. D.; Smith, P. J.; Chibale, K.; Smith, G. S. *Organometallics* **2012**, *31*, 5791.
23. Miernicka, M.; Szulawska, A.; Czyz, M.; Lorenz, I.-P.; Mayer, P.; Karwowski, B.; Budzisz, E. *J. Inorg. Biochem.* **2008**, *102*, 157.
24. Anderson, C. M.; Crespo, M.; Jennings, M. C.; Lough, A. J.; Ferguson, G.; Puddephatt, R. J. *Organometallics* **1991**, *10*, 2672.
25. Gómez, M.; Granell, J.; Martinez, M. *Inorg. Chem. Commun.* **2002**, *5*, 67.
26. López, C.; Solans, X.; Font-Bardía, M. *Inorg. Chem. Commun.* **2005**, *8*, 631.
27. Pérez, S.; López, C.; Caubet, A.; Bosque, R.; Solans, X.; Bardía, M. F.; Roig, A.; Molins, E. *Organometallics* **2004**, *23*, 224.
28. Pérez, S.; López, C.; Caubet, A.; Solans, X.; Font-Bardía, M. *New J. Chem.* **2003**, *27*, 975.
29. Crespo, M.; Font-Bardia, M.; Granell, J.; Martínez, M.; Solans, X. *J. Chem. Soc., Dalton Trans.* **2003**, 3763.
30. Capapé, A.; Crespo, M.; Granell, J.; Font-Bardía, M.; Solans, X. *J. Organomet. Chem.* **2005**, *690*, 4309.
31. Crespo, M.; Granell, J.; Solans, X.; Font-Bardia, M. *Organometallics* **2002**, *21*, 5140.
32. Ligands **2a** and **2b** hydrolyze in polar solvents (i.e., water, alcohols, DMSO) and this precluded the calculation of their IC_{50} values. Also, for the same reason it was imposible to determine for this two compounds the shift mobility of plasmid DNA.
33. Moradell, S.; Lorenzo, J.; Rovira, A.; Robillard, M. S.; Avilés, F. X.; Moreno, V.; de Llorens, R.; Martinez, M. A.; Reedijk, J.; Llobet, A. *J. Inorg. Biochem.* **2003**, *96*, 493.

34. Martin, R.; Crespo, M.; Font-Bardia, M.; Calvet, T. *Organometallics* **2009**, *28*, 587.
35. Cortés, R.; Crespo, M.; Davin, L.; Martín, R.; Quirante, J.; Ruiz, D.; Messeguer, R.; Calvis, C.; Baldomà, L.; Badia, J.; Font-Bardía, M.; Calvet, T.; Cascante, M. *Eur. J. Med. Chem.* **2012**, *54*, 557.
36. Chang, T. T.; More, S. V.; Lu, N.; Jhuo, J.-W.; Chen, Y.-C.; Jao, S.-C.; Li, W.-S. *Bioorg. Med. Chem.* **2011**, *19*, 4887.
37. El-Mehasseb, I. M.; Kodaka, M.; Okada, T.; Tomohiro, T.; Okamoto, K.-I.; Okuno, H. *J. Inorg. Biochem.* **2001**, *84*, 157.
38. Okada, T.; El-Mehasseb, I. M.; Kodaka, M.; Tomohiro, T.; Okamoto, K.; Okuno, H. *J. Med. Chem.* **2001**, *4*, 4661.
39. Ruiz, J.; Rodríguez, V.; Cutillas, N.; Espinosa, A.; Hannon, M. J. *J. Inorg. Biochem.* **2011**, *105*, 525.
40. Samouei, H.; Rashidi, M.; Heinemann, F. W. *J. Organomet. Chem.* **2011**, *696*, 3764.
41. Edwards, G. L.; Black, D. S. C.; Deacon, G. B.; Wakelin, L. P. G. *Can. J. Chem.* **2005**, *83*, 969.
42. Edwards, G. L.; Black, D. S. C.; Deacon, G. B.; Wakelin, L. P. G. *Can. J. Chem.* **2005**, *83*, 980.
43. Wang, P.; Leung, C.-H.; Ma, D.-L.; Sun, R. W.-Y.; Yan, S.-C.; Chen, Q.-S.; Che, C.-M. *Angew. Chem., Int. Ed.* **2011**, *50*, 2554.
44. Ma, D.-L.; Che, C.-M. A. *Chem. Eur. J.* **2003**, *9*, 6133.
45. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.
46. Matito, C.; Mastorakou, F.; Centelles, J. J.; Torres, J. L.; Cascante, M. *Eur. J. Nutr.* **2003**, *42*, 42.
47. Givens, K. T.; Kitada, S.; Chen, A. K.; Rothschiller, J.; Lee, D. A. *Invest. Ophthalmol. Vis. Sci.* **1856**, *1990*, 31.
48. Abdullah, A.; Huq, F.; Chowdhury, A.; Tayyem, H.; Beale, P.; Fisher, K. *BMC Chem. Biol.* **2006**, *6*, 3.
49. Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.
50. Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.
51. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O. A.; Austin, J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; González, C.; Pople, J. A. GAUSSIAN *03, (Revision C.02)*; Gaussian: Wallingford, CT, 2004.
52. Wadt, W. R.; Hay, P. J. *J. Chem. Phys.* **1985**, *82*, 284.
53. Hay, P. J.; Wadt, W. R. *J. Chem. Phys.* **1985**, *82*, 299.
54. Höllwarth, A.; Böhme, M.; Dapprich, S.; Ehlers, A. W.; Gobbi, A.; Jonas, V.; Köhler, K. F.; Stegmann, R.; Veldkamp, A.; Frenking, G. *Chem. Phys. Lett.* **1993**, *208*, 237.
55. Hehre, W. J.; Ditchfield, K.; Pople, J. A. *J. Chem. Phys.* **1972**, *56*, 2257.
56. Hariharan, P. C.; Pople, J. A. *Theor. Chim. Acta* **1973**, *28*, 213.
57. Cossi, M.; Rega, N.; Scalmani, G.; Barone, V. *J. Comput. Chem.* **2003**, *24*, 669.